STABLE HETERODIMERIC ANTIBODY DESIGN WITH MUTATIONS IN THE FC DOMAIN

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of U.S. application Ser. No. 15/409,456, filed Jan. 18, 2017, which is a continuation of U.S. application Ser. No. 13/289,934. filed Nov. 4, 2011, which claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Patent Application No. 61/410,746, filed Nov. 5, 2010; U.S. Provisional Patent Application No. 61/425,375, filed Dec. 21, 2010; U.S. Provisional Patent Application No. 61/439,341, filed Feb. 3, 2011; U.S. Provisional Patent Application No. 61/475,614, filed Apr. 14, 2011; U.S. Provisional Patent Application No. 61/491,846, filed May 31, 201 land U.S. Provisional Patent Application No. 61/497,861, filed Jun. 16, 2011, each of which is herein incorporated by reference in its entirety.

STATEMENT REGARDING SEQUENCE LISTING

[0002] This application incorporates by reference a Sequence Listing submitted with this application as text file Zymeworks V84467WO.txt created on Nov. 4, 2011 and having a size of 15 kilobytes.

FIELD OF THE INVENTION

[0003] The present disclosure generally provides polypeptide heterodimers, compositions thereof, and methods for making and using such polypeptide heterodimers. More specifically, the present invention relates to thermo-stable multispecific, including bispecific, antibodies comprising a heterodimeric Fc domain.

BACKGROUND OF THE INVENTION

[0004] Bispecific antibodies are antibody-based molecules that can simultaneously bind two separate and distinct antigens (or different epitopes of the same antigen). One use of bispecific antibodies has been to redirect cytotoxic immune effector cells for enhanced killing of tumor cells, such as by antibody dependent cellular cytotoxicity (ADCC). In this context, one arm of the bispecific antibody binds an antigen on the tumor cell, and the other binds a determinant expressed on effector cells. By cross-linking tumor and effector cells, the bispecific antibody not only brings the effector cells within the proximity of the tumor cells but also simultaneously triggers their activation, leading to effective tumor cell-killing. Bispecific antibodies have also been used to enrich chemo- or radiotherapeutic agents in tumor tissues to minimize detrimental effects to normal tissue. In this setting, one arm of the bispecific antibody binds an antigen expressed on the cell targeted for destruction, and the other arm delivers a chemotherapeutic drug, radioisotope, or toxin.

[0005] A major obstacle in the general development of bispecific antibodies has been the difficulty of producing materials of sufficient quality and quantity for both preclinical and clinical studies.

[0006] Traditional production of full-length bispecific antibodies is based on the co-expression of two immunoglobulin heavy chain-light chain pairs, where the two chains have different specificities (Millstein et al., 1983, Nature, 305:537-539). The intrinsic tendency of the Fc portion of the antibody molecule to dimerize leads to the formation of complex mixtures of up to 10 different IgG molecules consisting of various combinations of heavy and light chains, of which only one has the correct bispecific structure. Purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829, and in Traunecker et al., 1991, EMBO J., 10:3655-3659. Thus, the production of a bispecific antibody molecule with the two Fab arms selected to bind two different targets using traditional hybridoma techniques is challenging [Segal D M et al. (2001) J Immunol Methods. 248, 1-6.]. The trifunctional antibody, Catumaxomab, is a rat/mouse quadroma derived bispecific mAb and the purification of this molecule is achieved on the basis of a pH dependent elution in protein A column based chromatographic separation [Lindhofer H. et al. (1995) J Immunol 155, 219-225].

[0007] Another traditional method for bispecific antibody production is chemical conjugation of two antibodies or their fragments having different specificities. However, this method is also complicated, and the chemical modification process may inactivate the antibody or promote aggregation. Because purification from undesired products remains difficult, the resulting low yield and poor quality of bispecific antibody make this process unsuitable for the large scale production required for clinical development. In addition, these molecules may not maintain the traditional antibody conformation.

[0008] Recently, various heterodimerization techniques have been used to improve the production of bispecific antibodies. However, fusion of simple heterodimerization domains like the Jun/Fos coiled-coil to scFv domains lead to a mixture of homo- and heterodimers and need to be assembled by refolding (de Kruif and Logtenberg, J. Biol. Chem. 271: 7630-4, 1996). Fusion of scFv fragments to whole antibodies was also used as a dimerization device (Coloma and Morrison, Nat. Biotechnol. 15: 159-63, 1997). However, such fusion results in a large molecule with poor solid tissue penetration capabilities. Fusion of two scFv fragments together has also been used to generate bispecific proteins (e.g., BITE® antibodies by Micromet Inc., Bethesda, Md., U.S. Pat. No. 7,635,472). However, such proteins do not contain Fc regions, and thus do not allow manipulation of their activities via Fc regions. In addition, these proteins are small (~55 kDa) and thus have a relatively short half-live in serum.

[0009] In other heterodimerization techniques, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. This approach is disclosed in WO 94/04690. For further details of generating bispecific antibodies see, for example, Suresh et al., 1986, Methods in Enzymology, 121:210.

[0010] According to another approach described in WO96/27011, a pair of antibody molecules can be engineered to maximize the percentage of heterodimers that are